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Use of Bioassays to Determine Potential Toxicity Effects of Environmental Pollutants

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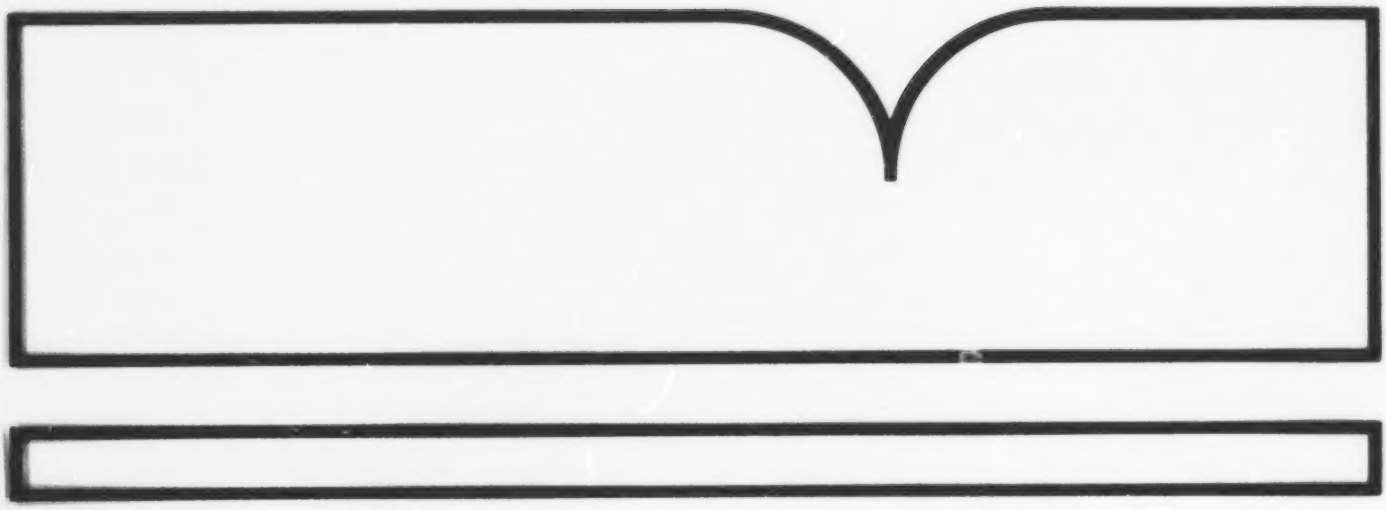

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USE OF BIOASSAYS TO DETERMINE POTENTIAL TOXICITY EFFECTS OF ENVIRONMENTAL POLLUTANTS

by

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USE OF BIOASSAYS TO DETERMINE POTENTIAL TOXICITY EFFECTS OF ENVIRONMENTAL POLLUTANTS

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ABSTRACT

Nonpoint source (NPS) runoff from mining, landfills, roads, croplands, grazing lands, and forests can contain chemicals harmful to aquatic organisms. Full scale biological surveys to determine their effects are difficult and costly. Bioassays of environmental samples integrate the effects of all toxicants contained in a sample. Biological organisms are being used more frequently to identify toxicant problems and to rank-order their severity. The Corvallis Environmental Research Laboratory (CERL) has developed a multi-media (aquatic/terrestrial) bioassessment protocol to assist in the identification of toxicity potentials associated with waste disposal. Similar techniques can be used to identify NPS pollutants. The bioassay response indicators are particularly useful in identification of field-site problems where complex mixtures of pollutants might be present. Use of the bioassessment protocol reduces the initial need for extensive chemical analyses, and produces data (toxicity LC_{50} information) in a form more readily understood by the public than bulk chemical concentrations. The CERL protocol has been used successfully to: (1) define

and rank-order the effects of selected heavy metals, herbicides, and insecticides on microbes, earthworms, plant seeds, algae, daphnia, and fathead minnow larvae; (2) determine that rank-order of sensitivity differs with major toxicant groups; (3) detect the presence of bioactive organic and heavy metal mixtures in field site samples when concentrations of priority organic pollutants did not exceed EPA criteria levels; and (4) identify the basic chemical component of complex waste mixtures which produce environmental toxicological effects. These types of information should be useful in determining the potential effects of NPS pollutants and in designing measures for their control.

INTRODUCTION

Nonpoint sources (NPS) pollution problems are among the most pervasive, persistant, and diverse water quality problems facing the nation. This presents a definite problem to water quality decision makers who traditionally have addressed individual pollutants or site specific sources of pollutants. The individual chemical by chemical approach requires a great deal of patience, time, money, and intellect to determine the pollutants adverse impact. Also, determining the substance producing the impact, the source of the substances, and the areal extent of the problem is difficult to address. Even extensive effort on a chemical by chemical basis does not assure an accurate ecotoxicological assessment, since one still has to relate environmental chemical measurements to a biological/ecological impact. The approach most commonly employed is that of calculating potential toxicity based on chemical concentration of the 129 EPA consent decree chemicals (priority pollutants) (Keith and Telliard, 1979) with extrapolation to water quality criteria. The approach has been useful in providing relative toxicity guidance, i.e., the relative toxicity of various chemicals under laboratory conditions. However, it has become increasing apparent that this approach has severe limitations concerning realistic and accurate ecotoxicity estimates. Some of the problems associated with calculation of toxicity potentials based on priority pollutant chemical concentrations are that:

1. the data bases for most chemicals are not complete enough to permit the development of reliable criteria;

2. most of the chemicals for which complete criteria exist are not necessarily those most commonly found in complex NPS or waste site discharges;
3. application of criteria to field situations usually result in highly conservative and, therefore, overly restrictive estimates of toxicity or misinterpretation of toxicity cause and effect relationships;
4. criteria for single chemicals were not intended to be assembled additively and there is little evidence to support that use; and
5. for contaminated soil and sediment there are no criteria on which to base decisions for judging if a site constitutes a problem.

Biological assessment of environmental toxicity alleviates most concerns associated with the above problems and provides a direct indication of potential toxicity (Roop and Hunsaker, 1985). An example was cited by Samoiloff et al. (1983) when they discovered that the most toxic sediment samples were those containing none of the EPA consent decree chemicals. Miller et al. (1985) have demonstrated similar results with the bioassessment of hazardous waste site samples using a multimedia bioassay procedure. Brown et al. (1984) demonstrated the inability of chemical analyses to provide a comprehensive evaluation of the toxicity potential of hazardous industrial wastes. They demonstrated further that a combined testing protocol using bioassays and organic chemical analysis was effective in identifying the toxicity potential of such wastes. A recommendation from Brown et al. (1984) was that a battery of bioassays be used to define the toxicity of wastes. The purpose of this paper is to demonstrate that such a bioassay test battery and analysis of results can be used to: (1) identify and rank-order toxicity hazard potential of waste site samples; (2) help define and quantify areal extent of toxicity potentials; (3) help identify what chemical fractions of a complex waste contribute significantly to their overall toxicity; and (4) suggest that similar procedures

might be used to assess the impacts of a broad spectrum of NPS pollutants. This paper is based, in part, on recently published and ongoing research conducted or sponsored by the Hazardous Materials Assessment Team at the EPA Corvallis Environmental Research Laboratory.

METHODS

Biological organisms respond to the adverse effects of a variety of specific pollutants (FWPCA, 1968 and USEPA, 1976). However, there has been relatively little comparative toxicology done on environmental samples using a broad spectrum of organisms comprising both aquatic and terrestrial compartments of the ecosystem. For this purpose, we have adopted a multi-media bioassessment protocol described by Porcella (1983). The bioassays in the Porcella protocol include assessments of water and soil leachate toxicity on seed germination/root elongation (lettuce, Lactuca sativa L.), earthworms (Eisenia foetida), algae (Selenastrum capricornutum), daphnia (Daphnia magna), and fathead minnow larvae (Pimephales promelas). In addition, we have conducted Microtox (Photobacterium phosphoreum) tests (Beckman, 1982). Our approach has been to conduct comparative toxicological studies on pure chemicals and mixtures of chemicals in the laboratory to increase our confidence that biological responses to these substances are predictable and relatable to environmental samples (Miller et al., 1985). All toxicity responses are expressed as EC_{50} or LC_{50} concentrations for comparison.

We have focused on substances in chemical extraction groupings. Metals, base neutral organics, acid organics, and pesticides were extracted with water (4 ml water to 1 g soil). Bioassays were performed using these aqueous extracts. The predicted bioassay response, based on chemical concentration and criteria for certain chemicals, was then compared with bioassay responses on

environmental samples dominated by the mixture of chemicals in question. This approach has permitted us to test the hypothesis that bioassay of environmental samples will produce EC_{50} or LC_{50} estimates significantly different from those predicted by calculation based on chemical concentrations with extrapolation to water quality criteria. Also, we have examined the relative toxicity potential of various metals, priority organics, and nonpriority organics in samples, from the Western Processing Superfund site at Kent, Washington. This was accomplished by incremental inactivation of metals with EDTA (at an EDTA to metals molar ratio of 4:1, based on Cu inactivation) and methylene chloride extraction of priority organic chemicals (Eichelberger et al., 1983) followed by algal assay examination. Chemical quality control was assured by surrogate spike recovery analysis coupled with daily calibration of the GC/MS system.

Extent of chemical contamination was determined using a modified phytotoxicity test described by Thomas and Cline (1985). Lettuce seeds were used to test the toxicity potential of soils collected along four, 90 m long parallel transects that were 15 m apart. Soils from 0-15 cm depth and 15-30 cm depth were used since they encompassed the root zone in the area. The site was located downwind, along a suspected concentration gradient perpendicular to an open ditch known to have transported liquid organic wastes associated with the manufacture of herbicides, insecticides, and neurotoxin gases at Rocky Mountain Arsenal, Colorado. Thomas et al. (1984) have described the statistical sampling design in greater detail. Phytotoxicity data from the site were analyzed using kriging. Kriging is a statistical technique developed in the mining industry (Clark, 1982). Only a limited number of samples are required to successfully define a contaminated area using kriging. The technique employs a weighted moving average that calculates point estimates or block averages over

a specified grid. Output of the kriging analysis for this study is a contour map displaying areal variation in phytotoxicity.

FINDINGS AND DISCUSSION

Comparative Toxicology

Miller et al. (1985) conducted comparative toxicological studies on several known single and complex organic and metal contaminants in the laboratory using the Porcella (1983) bioassessment protocol plus the Microtox Test (Beckman, 1982). They concluded that:

1. the protocol test organisms responded differentially to various pollutants and their EC_{50} or LC_{50} results generally conformed to the range of values reported in the literature for individual chemicals and metals;
2. test organism rank order of sensitivity differed with major toxicant groups suggesting that certain bioassays are better suited than others to assess given chemical groups;
3. algae (Selenastrum capricornutum) was the most uniformly sensitive test organism across a broad spectrum of pollutant groupings; and
4. differences in sensitivity levels of the test organisms, relative to the toxicant assayed, can be used to identify those biotic components most susceptible to the presence of toxicants and to draw an educated conclusion as to the contaminant type producing the toxic effect.

Based on the above conclusions drawn from bioassay responses to pure chemical substances in the laboratory and the assumption that bioassays integrate the toxicity effects of all sample components regardless of their composition, Miller et al. (1985) bioassayed soil and soil elutriate samples from seven diverse hazardous waste sites (Table 1). The samples were dominated by heavy metals, solvents, phthlates, phenols, pesticides, and herbicides.

Table 1. EC_{50} response for (%) in soil (earthworm) or soil elutriate with associated complex chemical contaminants from selected hazardous waste sites.

Waste Site	Major Chemical Group	Bioassay Response (Percent of soil or elutriate required to produce an EC_{50}) Algae	Daphnia	Microtox	RE1	Earth-worm	Arith-metic Average
Holder Chemical West Virginia	Pesticides, herbicides	2.1	3.6	18.0	3.6	70.0	19.5
Western Processing Kent, WA #17	Heavy metals, phenols, solvents, pesticides	0.2	5.6	2.2	37.0	55.0	20.0
Big John Houldt West Virginia	PAH3, unknown organics	5.4	87.0	28.0	NE4	<10.0	46.1
Hollywood Memphis, TN	Pesticides	24.0	22.0	>90.0	NE	>25.0	52.2
Sharon Steel Fremont, NY	Heavy metals, tar, PAH	0.6	30.0	99.0	NE	>75.0	40.4
Sapp Battery Cottondale, FL	Heavy metals	41.0	70.0	NE	NE	NE	22.2
Thiokol Chester, WV	Diphenylamine	NE	NE	NE	NE	35.0	87.0

1 Root elongation test.

2 Earthworm 14 day soil contact test (LC_{50}).

3 PAH = polynuclear aromatic hydrocarbons.

4 NE = No effect observed at 100% of the soil or soil elutriate. Therefore, NE is factored into the arithmetic average as 100%, i.e., 100% of the soil or elutriate produced no effect on the test organisms; the greater the percent soil or elutriate required to produce the EC_{50}, the less toxic is the sample.

Relative, integrated biotic toxicity of the sites and their rank ordering could be determined by calculating the arithmetic average toxicity across the different tests in Table 1. If one was concerned primarily with potential aquatic impacts, the algae, Daphnia, and Microtox tests probably would be the most applicable indicators. The sensitivity of algae appears to be much greater than the other bioassays for most of the samples. Algae responded adversely to all but one of the samples. In that case, no aquatic test responded adversely. Toxicity rank ordering, such as that shown in Table 1, would be helpful in: (1) determining potential environmental impacts; (2) directing further chemical analyses within sites; and (3) prioritizing cleanup across or within various sites. Bioassay data might be used to monitor toxicity changes in samples before and after waste cleanup or the adoption of various NPS management alternatives, thus helping to determine the degree of treatment success.

Kriging of Bioassay Data

Another means of assimilating bioassay data into a format useful for problem solving and remedial design relative to chemical hazard assessment is that of kriging. Phytoassay responses for soil samples from Rocky Mountain Arsenal were subjected to kriging as described under methods. Kriging the 0-15 cm phytotoxicity data, with the resultant toxicity potential contours is shown in Figure 1.

Thomas et al. (1984) compared kriged phytotoxicity bioassay estimates (Figure 1) with sample site specific plant mortality data (Figure 2). This type of graphic interpolation could be very useful in making waste site cleanup decisions or in designing NPS watershed or ecoregional contaminant source controls. For example, if it was determined that the 30 percent mortality

contour should be used as the criterion for remedial action for the conditions shown in Figure 2, the area below the 30 percent solid contour line would be targeted for action. Unfortunately, the hazardous waste site situation is more complex than the kriged phytotoxicity surface data (0-15 cm deep) would indicate. Samples from the 15-30 cm depth at the same site produced the results shown in Figure 3. Comparison of kriging estimates with plant mortality data at this depth is shown in Figure 4. It is evident that site cleanup based on the surface sample greater than 30 percent mortality results would omit significant areas of contamination. This information makes the remedial action plan more complicated, but it adds significant realism to the site assessment. A final remedial action decision that includes consideration of chemical bioavailability as determined by integrative bioassay endpoints should greatly enhance the probability of contaminant cleanup success. Chemical information alone cannot assure an accurate assessment of toxicity potentials and in some instances might lead to misinterpretation of toxicological cause and effect relationships.

Chemical Analysis and Bioassay

Hazardous waste assessment and NPS pollution problems are similar in that each has traditionally been assessed from a chemical perspective. Severity of the problem has been assessed relative to the concentration of a given chemical producing a given type and degree of response under laboratory conditions. Controlled condition Laboratory response tests have been used extensively to develop water quality criteria for various chemicals. Problems associated with the extrapolation of these criteria to assess field conditions were mentioned in the introduction. In addition, combinations of pollutants and different

attenuating characteristics of a site are difficult to assess when calculating toxicity estimates.

Direct bioassay of samples tend to minimize many of these problems. Bioassays integrate the toxicological effects of all sample components regardless of their type and amount. Chemical presence is of limited concern, but bioavailability of the chemical and its effect on the test organism is of great concern. Bioassay of the waste samples provides a direct estimate of its toxicity potential.

Hazardous waste cleanup decisions have relied heavily on analysis of the EPA 1965 consent decree chemicals (the so called 129 priority pollutants). Concentrations of these pollutants in excess of water quality criteria values have been used to justify various cleanups, but in many instances environmental criteria do not exist. In these cases the chemical information may be more misleading than it is helpful since one suspects there may be some hazard, but there is little information for determining the degree of hazard based on the chemical analysis.

Herein lies the benefit of the bioassay procedure. Soil and water bioassays in the Porcella (1983) bioassessment protocol will provide an indication of toxicity to various compartments of the system. Also, it will provide a quantitative (EC_{50} or LC_{50}) ranking of the toxicological impact potentials among those compartments.

We believe that reliance on chemical criteria alone, and particularly those for priority pollutants, could lead to erroneous decisions concerning remedial actions. The general chemical analytical protocol for hazardous waste site samples calls for priority metal and organic identification and quantification. In some instances the next ten most prominent GC/MS peaks beyond the priority organics might be "identified." Data bases for many of these pollut-

ants are too limited to allow one to develop rigorous water quality criteria. This is especially true for nonpriority organics. Where toxicity data are not available, it might be necessary to "estimate" the potential toxicity of chemicals based on their similarity to other chemicals for which toxicity data does exist. This introduces yet another uncertainty factor. At present there seems to be no satisfactory method of estimating toxicity for organic contaminants short of direct bioassay of environmental samples.

Figure 5 illustrates how difficult it might be to estimate environmental toxicology or the cause of toxicity based on chemical analyses of priority pollutants. The figure represents a typical GC/MS scan of a waste site sediment leachate sample. Results in Table 2, with the exception of the onsite ponded water, represent sediment leachates from an off-site reference control (East Ditch), an onsite reference (005, thought to be uncontaminated) and two off-site stream sediment samples (017 downstream and 020, upstream). Sample 005 contained four identifiable priority organics, nine identifiable nonpriority organics, and fourteen unidentifiable nonpriority organic substances.

Concentrations of phthalates, ethylbenzene, nitrosamines, and phenol priority pollutant fractions for the various samples collected at Western Processing are shown in Table 2. The table also shows the nonpriority organic fractions and the total organics. Among the four identifiable priority pollutants, an environmental criteria exists only for phenol (3.4 mg/l). Assuming that priority pollutant concentrations are among the most important consideration of hazard potential at a site and that water quality criteria are paramount in assessing hazard potentials, sample 017 should be highly toxic due to the presence of phenol at a concentration of 18.3 mg/l. Chemical concentrations and water solubilites of the other priority pollutants would suggest that the other samples might be nontoxic. Bioassay of the samples did not support

Table 2. Concentration (mg/l) of priority and nonpriority organics contained in sediment leachates from Western Processing (modified from Miller et al., 1985).

Constituent	Control	East Ditch Water	Pond 005	017	020
Phthalates	0.002	0.010	0.001	0.011	0.002
Ethylbenzene	0.002	<0.001	0.001	0.001	0.001
Nitrosamines	<0.001	<0.001	<0.001	0.010	<0.001
Phenol	<0.001	<0.001	<0.001	18.370	<0.001
Nonpriority Organics	0.3	15.9	0.8	11.9	0.2
Total Organics	0.3	15.9	0.8	29.9	0.2

this conclusion (Table 3). Comparing the mean EC_{50} or LC_{50} value for the different test organisms it can be seen that the toxicity of sample 017 was quite similar to the East Ditch Control sample. The upstream reference sample was not toxic. The onsite ponded water was highly toxic as was sample number 005 (thought to be uncontaminated). Toxicity of the samples increased as the nonpriority organic fractions increased.

To test the apparent relationship between toxicity and the nonpriority organic component of the Western Processing Samples we conducted algal assays on 0-1.0 m integrated soil core samples taken on site at locations 1, 11, and 17 (the latter should not be confused with sediment sample 17 above). Results of the algal assays are shown in Figure 6. The results indicated that soil cores from site 17 were the most toxic and that toxicity increased across the three samples as the concentrations of soluble metals, soluble priority organics, and total soluble organics increased. It was not readily apparent from this which toxic component was dominant in the system.

There was some evidence that toxicity increased with depth in the soil column (not shown in this data). Therefore, we elected to use leachate from the 3 m (integrative depth from 2-3 m) depth at site 17 to further evaluate the toxic components of the samples. Bioassays were run sequentially on untreated sample, EDTA chelated sample (metals inactivated) and on combined chelation/ priority organic extracted (methylene chloride) sample. Results show that chelation of soluble metals with EDTA decreased toxicity 90 fold, but that the chelated elutriate remained highly toxic (Figure 7). Significant additional toxicity reduction was not realized when the sample was subjected to combined chelation and priority organic extraction. It appears from this analysis that metal toxicity dominated the Western Processing samples, but that nonpriority organic chemicals alone were sufficient to classify the soil leachate as highly

Table 3. EC_{50} or LC_{50} response in % sediment[1] (earthworm), sediment elutriate, and surface water to chemical contaminants in Western Processing Samples (modified from Miller et al., 1985).

Test Organism	East Ditch Control	Pond Water	005	017	020
Algae	45	0.8	0.4	24.9	NE2
Daphnia	90	18.5	3.3	NE	NE
Microtox 5 min	NE	82.7	41.2	55.4	NE
15 min	NE	21.3	<5.6	50.1	NE
30 min	NE	10.2	<5.6	43.4	NE
Lettuce RE	NE	NE	61.4	49/1003	NE
Earthworm[4]	NE	--	>50<100	>100	--
Mean EC_{50}[5]	87.0	32.4	34.1	73.7	>100

1 Sediment soil samples.

2 NE = no significant toxicity was observed.

3 49/100 = 49% inhibition in 100% sediment elutriate.

4 LC_{50} values = concentration at which 50% mortality occurs.

5 Mean of algae, daphnia, microtox 30 min, lettuce RE, and earthworm tests.

toxic. The toxicological influence of priority organics in these samples appears to have been minimal. Therefore, predicted toxicity of these samples based on the concentration of priority pollutants would have severely underestimated sample toxicity.

SUMMARY

We have attempted to develop a biological toxicity screening protocol that has broad based application potential. Based on results to date we believe:

1. A modified Porcella bioassessment protocol can be used to define and rank order the effects of selected heavy metals, herbicides, and insecticides.
2. It appears that selected segments of the protocol can be used to assess the influence of complex wastes under field conditions, i.e., there is a relationship between laboratory bioassay responses to environmental samples and actual field conditions.
3. The protocol can be used to assess environmental toxicity potentials in situations where water quality criteria are lacking or nonexistent.
4. Direct bioassay of environmental samples produces toxicity results significantly different from those predictions based on measured chemical concentrations with extrapolation to water quality criteria.
5. Experience gained from the bioassay of hazardous waste site samples should have application to many aspects of the NPS pollution problem.
6. Algal assay appears to have great universal toxicant/stimulant assessment potential based on sensitivity to various toxicants.

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